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BENEFICIAL ARTHROPOD BEHAVIOR MEDIATED BY AIRBORNE SEMIOCHEMICALS. IX. DIFFERENTIAL RESPONSE OF *Trichogramma pretiosum*, AN EGG PARASITOID OF *Heliothis zea*, TO VARIOUS OLFACTORY CUES

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Abstract—The behavior of *Trichogramma pretiosum* Nixon wasps when exposed to different olfactory cues was studied in a wind tunnel. Compared to clean air, the sex pheromone of its host *Heliothis zea* (Boddie) increased wasp residence times, walking times, and path lengths on a platform and decreased walking velocity. If wasps were released on top of a glass rod above a platform, the odor caused the wasps to land shortly after takeoff. In addition, a clear dose effect with regard to total residence and walking times was found. These responses were not elicited by three dosages of the sex pheromone of *Spodoptera frugiperda* (J.E. Smith) or by a blend of saturated acetates. These results correspond with the observation that *H. zea* is a common field host of *T. pretiosum*, whereas eggs of *S. frugiperda* are rarely attacked by this parasitoid.

Key Words—*Trichogramma pretiosum*, Hymenoptera, Trichogrammatidae, *Heliothis zea*, *Spodoptera frugiperda*, Lepidoptera, Noctuidae, sex pheromone, kairomone, wind tunnel, orientation behavior.

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INTRODUCTION

Olfactory receptor systems of insects are shaped continuously by sensory adaptation and natural selection to perceive chemical cues (i.e., semiochemicals) in an ever-changing environment. Of particular significance for foraging parasitic wasps are host-derived kairomones, the function of which has now been demonstrated for many species of parasitoids in a variety of taxonomic groups (Vinson, 1984, 1985; Noldus, 1989c). Here we focus on cases where the kairomone used by a parasitoid is part of the intraspecific communication system of the host, so that the parasitoid can be labeled as a "chemical eavesdropper" or "chemical spy." Examples are known from several parasitoids of bark beetles that respond to their host's aggregation pheromone (Wood, 1982). A similar phenomenon has been demonstrated recently for egg parasitoids of the genus *Trichogramma*. These insects have received—and continue to receive—considerable research attention, due mainly to their potential as biological control agents against lepidopteran crop pests (Voegelé et al., 1988). *Trichogramma pretiosum* Riley, for example, is a candidate for inundative releases against *Heliothis* spp. (King et al., 1986; Ridgway et al., 1988; King and Coleman, 1989).

Initial field and greenhouse observations (Lewis et al., 1982) and subsequent olfactometer experiments (Noldus, 1988) indicated that female *T. pretiosum* utilize the sex pheromone of *H. zea* as a kairomone in their search for host eggs. We recently analyzed the orientation behavior of two *Trichogramma* spp. (*T. evanescens* Westwood and *T. pretiosum*) in response to the sex pheromone of their noctuid hosts [*Mamestra brassicae* L. and *Heliothis zea* (Boddie)] in a wind tunnel (Noldus et al., 1990a). Orientation by wasps to host sex pheromone was achieved by a combination of odor-modulated phototaxis and odor-induced inverse orthokinesis. Compared to clean air, kairomone-loaded air decreased walking velocity and increased residence times, walking times, and path lengths on a platform. During locomotion on a horizontal platform, upwind anemotaxis was evident but remained unaffected by odor. On a platform inclined 45°, the lower edge facing upwind, anemotaxis appeared to be offset by positive phototaxis. If wasps were released on top of a glass rod above a platform, the kairomone caused wasps to land shortly after takeoff.

The sex pheromone of *H. zea* is a mixture of four straight-chain even-carbon-numbered aldehydes. The synthetic blend identified as the most effective sex attractant in the field consisted of (*Z*)-11-hexadecenal (Z11–16 : Al), (*Z*)-9-hexadecenal (Z9–16 : Al), (*Z*)-7-hexadecenal (Z7–16 : Al) and hexadecanal (S–16 : Al), in a 87 : 3 : 2 : 8 ratio (Klun et al., 1980). This blend also was used in the previous experiments with *Trichogramma* (Lewis et al., 1982; Noldus et al., 1989a). Given the fact that *T. pretiosum* is found on several species of Lepidoptera in the field (Pinto et al., 1986), one may wonder if *T. pretiosum*

will respond to any randomly chosen blend of straight-chain aliphatic compounds.

Further, the responses of *T. pretiosum* to the sex pheromone of *H. zea* recorded so far were obtained in laboratory setups, either a four-arm airflow olfactometer (Noldus, 1988) or a wind tunnel (Noldus et al., 1990a), in which host pheromone was tested against clean air. In the absence of other stimuli for comparison, responses to stimuli recorded in such assays might represent a response to something vs. nothing, rather than a host-directed response (Jones, 1986).

To investigate these two hypotheses, comparative wind tunnel experiments were carried out in which *T. pretiosum* was exposed to the sex pheromone blend of *H. zea* and to (1) a biologically active sex pheromone blend of another moth species, but nonhost of *T. pretiosum*, and (2) an artificial blend of acetates.

As the first test substance, we used the sex pheromone of the fall armyworm moth, *Spodoptera frugiperda* (J.E. Smith). This was chosen because both *S. frugiperda* and *H. zea* are noctuids, with similar geographic distributions and substantial overlap in host-plant ranges (Harding, 1976; Sparks, 1979; Hill, 1987). However, *S. frugiperda* is not a host of *T. pretiosum*. Calling female *S. frugiperda* release a five-component blend, consisting of (*Z*)-7-dodecen-1-yl acetate (Z7–12 : Ac) (3.2%), 11-dodecen-1-yl acetate (11–12 : Ac) (22%), dodecan-1-yl acetate (S–12 : Ac) (1.9%), (*Z*)-9-tetradecen-1-yl acetate (Z9–14 : Ac) (90.1%) and (*Z*)-11-hexadecen-1-yl acetate (Z11–16 : Ac) (2.6%) (Tumlinson et al., 1986).

The second test material was a mixture that was chemically related to lepidopteran sex pheromones but that *T. pretiosum* would not encounter in the field. We used a blend of three saturated acetates—dodecanyl acetate (S–12 : Ac), tetradecanyl acetate (S–14 : Ac) and pentadecanyl acetate (S–15 : Ac)—that has no known function as a semiochemical.

METHODS AND MATERIALS

Parasitoid Rearing. Trichogramma pretiosum wasps were reared on eggs of *H. zea* as described by Noldus (1988). *Heliothis* eggs were obtained from a laboratory culture; they had been treated with a solution of sodium hypochlorite for removal of adhesives and other chemicals and for sterilization (Burton, 1969). Only 2-day-old female wasps were used. To reduce interindividual behavioral variation they were allowed to oviposit in a *H. zea* egg ca. 1 hr prior to the experiment and were then isolated for ca. 30 min (Noldus, 1988; 1989b).

Odor Sources. The following odor sources were used in the experiments:

1. The synthetic sex pheromone of *H. zea*. The blend consisted of a 87 : 3 : 2 : 8 mixture of Z11–16 : Al, Z9–16 : Al, Z7–16 : Al and S–16 : Al. It was

loaded on rubber septa at dosages of 0.1, 1, and 10 mg. These high dosages were necessary to obtain release rates in a range covering that of a calling female, at an airflow of 500 ml/min over the septum. A septum loaded with 1 mg released ca. 55 ng/hr, as determined by collection of volatiles and analysis by gas chromatography using the method of Heath et al. (1986). This is close to the rate at which the major component Z11–16 : Al is released during peak emission (Pope et al., 1984).

2. The synthetic sex pheromone of *S. frugiperda*. The blend tested was loaded on rubber septa in such a ratio as to release the five compounds in the approximate ratio as they are released by calling females. It consisted of Z7–12 : Ac (0.6%), 11–12 : Ac (0.7%), S–12 : Ac (0.4%), Z9–14 : Ac (87.0%) and Z11–16 : Ac (11.3%), loaded on septa at dosages of 0.1, 1, and 3 mg.

3. A blend of three saturated acetates (S–12 : Ac, S–14 : Ac and S–15 : Ac) in a 33 : 33 : 34 mixture. This blend was tested at a dosage of 1 mg/septum.

The desired amount of a blend in 200 μl hexane was pipetted into the large well of a rubber septum and the solution was allowed to soak into the septum. Then the septum was aired under a laboratory hood for 48 hr to remove the solvent. Before a test, septa were aired for 1 hr, and between tests they were stored at room temperature.

Wind Tunnel Experiment. We used a horizontal wind tunnel similar to the one described elsewhere (Noldus et al., 1990a), with minor modifications. Air was blown over the septum at a rate of 500 ml/min, of which 70% was discarded via a three-way stopcock directly downwind of the container holding the septum. Odor was injected into the main stream by blowing the odor-laden air through a glass nozzle (made from a Pasteur pipet, cf. Zanen et al., 1989) at a rate of 150 ml/min. With the prevailing ventilator speed (which produced a main stream of ca. 5 cm/sec), this resulted in a turbulent jet plume with a diameter of ca. 10 cm and an airspeed of ca. 15 cm/sec in the center at 45-cm downwind from the opening of the nozzle (measured with a hot-wire anemometer). At this point, a platform was so located as to be completely enveloped by the plume. The design of experiments was based on a combination of overhead light and airborne chemicals as competing stimuli for *Trichogramma* wasps (see Noldus et al., 1989a). Overhead illumination was provided by four fluorescent tubes, which gave a light intensity of ca. 5.9 W/m^2 at the center of the platform.

At the start of a test, a septum loaded with test chemicals was placed in a brass container, which was connected to the nozzle, after which the airflow was started. Observations were begun after the pump had run for at least 20 min, to allow the odor concentration to stabilize. Treatments were alternated in a systematic fashion. Between treatments, the wind tunnel was rinsed with ethanol and clean air was drawn through at 50 cm/sec for at least 2 hr. After a day of testing, the wind tunnel was rinsed with ethanol and ventilated with clean air

until the following day. The behavior of the insects was recorded on a TRS-80 Model 100 microcomputer, programmed as an event recorder (cf. Noldus, 1990). All experiments were performed at 29 ± 1°C and 60 ± 10% relative humidity.

Walking Experiments. Two types of experiments were conducted. In both, platforms were used as experimental arena in order to mimic leaves. In the first, referred to as walking experiments, the experimental arena consisted of a platform, inclined at 45°, with the lower edge facing the wind. The center of the platform was 15 cm above the floor of the wind tunnel. It was made of white cardboard covered with translucent plastic and had a surface area of 5 × 5 cm. A thin streak of odorless petroleum jelly along the edge underneath the platform prevented the wasps from walking to the lower surface. The platform was cleaned with ethanol after every observation. Wasps were released individually in the center of the platform and were observed until they flew away or until 10 min had elapsed. The distance between the observer and the wasps hampered detailed inspection of their behavior, which was therefore classified as either walking or not walking. In order to record the position of the insect, a grid of 25 square sectors (1 × 1 cm) was drawn on the platform, and behavioral events were recorded separately for each sector. The following parameters were measured: total time on the platform, time spent walking, proportion of time spent on the upwind half of the platform, path length [estimated by counting the total number of platform sectors traversed (cf. Reddingius et al., 1983); as this parameter was only used for comparative purposes, no conversion factor was applied to estimate the distance actually covered], walking velocity (estimated total path length divided by absolute walking time), and number of different platform sectors traversed [when compared to the total number of sectors (25), this can be used as a crude index of the proportion of the platform traversed].

Flight Experiments. The second type of experiment, referred to as flight experiments, were designed to test the effect of various odors on *Trichogramma*'s behavior at the onset of flight. Wasps were released individually on top of a glass rod (4 mm diam.), extending 2 cm above the center of a green horizontal platform (6 × 6 cm), with a 2-mm-wide ring of odorless petroleum jelly around the rod (1 mm from the top) preventing wasps from walking down the rod. Flights could be categorized into upward flights towards the overhead light (which resulted in a landing on the ceiling or—very rarely—on the side wall of the tunnel), and flights resulting in a landing on the platform, providing an assay to test the effect of odor on: latency to takeoff, percentage of individuals landing on the platform, and frequency of landings on the upwind and downwind half of the platform. Parasitoids that did not take off from the rod within 5 min were discarded (ca. 1% of the trials). After every observation the rod was cleaned with ethanol to prevent accumulation of odor molecules or traces of the wasps.

Statistical Analysis. Frequencies and durations of behaviors were com-

puted with The Observer, a software package for behavioral research (Noldus, 1989a, 1990). All data were tested for normality, using the Shapiro-Wilk test (if $N \leq 50$) or the Kolmogorov test (SAS, 1985). The distribution of most parameters deviated significantly from a normal distribution. Those parameters were analyzed with nonparametric statistical tests. Otherwise parametric tests were used. In the dose–response experiments, the dosages were compared to test for a dose effect; in a number of cases values of a single dosage were tested against those obtained with clean air. Further details are given as footnotes in the tables.

RESULTS

Response to Various Olfactory Cues. In the first walking experiment, parasitoids were exposed to three different odors and clean air. All odors were loaded on septa at a dosage of 1 mg. As shown in Table 1, only the sex pher-

TABLE 1. BEHAVIOR OF INDIVIDUAL *Trichogramma pretiosum* WASPS ON PLATFORM IN WIND TUNNEL WHEN EXPOSED TO VARIOUS STIMULI[a]

Parameter	Stimulus: Clean air	Sex pheromone *Heliothis zea*	Sex pheromone *Spodoptera frugiperda*	Acetate blend	P^b
Number of observations	50	50	50	50	
Total time on platform (sec)	99.9 ± 15.6[c]a	186.3 ± 22.8b	101.0 ± 15.3a	73.7 ± 16.9a	**
Time spent walking (%)	71.5 ± 3.1a	86.3 ± 2.6b	72.5 ± 2.9a	78.2 ± 4.7a	**
Estimated path length (number of sectors traversed)	17.9 ± 2.8a	30.3 ± 3.4b	15.6 ± 1.6a	12.8 ± 1.4a	**
Walking velocity (number of sectors traversed/sec)	0.33 ± 0.02a	0.24 ± 0.02b	0.35 ± 0.02a	0.41 ± 0.02a	**
Number of different sectors traversed	11.2 ± 0.9a	15.7 ± 1.0b	10.9 ± 0.8a	9.0 ± 0.7a	**
Time spent in upwind half (%)	53.3 ± 4.4a	44.3 ± 3.5ab	35.4 ± 3.8b	45.9 ± 4.4ab	*
Wasps spending more than 50% time in upwind half (%)	58.0 (n.s.)[d]	38.0 (n.s.)	30.0 (**)	32.0 (*)	

[a] The platform measured 5 × 5 cm, and was divided into 25 1 × 1-cm sectors.
[b] Kruskal-Wallis test (SAS, 1985), followed by distribution-free multiple comparison (Hollander and Wolfe, 1973). Different letters in a row indicate significant differences. n.s.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.
[c] Mean ± SE.
[d] In parentheses: 2-tailed significance level of difference from 50% (sign test) (Siegel, 1956).

omone of *H. zea* elicited responses. These were typical of those found previously (Noldus et al., 1990a): increased total residence and walking times, path length, and proportion of platform traversed and reduced walking velocity. The only significant effect of the sex pheromone of *S. frugiperda* was a tendency for wasps to spend proportionally more time in the downwind half of the platform, compared to clean air.

Similar results were obtained in the first flight experiment (Table 2): unlike the sex pheromone of *H. zea*, the other odors did not alter the percentage of wasps landing on the platform. Latency and the frequency of landings on upwind and downwind halves of the platform remained unaffected by the odor offered. In no treatment did the percentage of individuals landing on a given side differ significantly from 50%.

Dose-Response Relationships. In the previous experiments, *T. pretiosum* responded to the sex pheromone of *H. zea* but not to that of *S. frugiperda*. However, both stimuli were compared at a single dosage, and a response to the fall armyworm sex pheromone might be obscured by a different dose-response relationship. The lack of response to the sex pheromone of *S. frugiperda* might thus have been due to the dosage tested. In addition, the sex pheromone of *H. zea* had been tested at ca. 30% of the concentration released by individual calling females. However, *T. pretiosum* is likely to be exposed to the kairomone in the field at a different concentration. Therefore, we examined the behavior

TABLE 2. FLIGHT BEHAVIOR OF *Trichogramma pretiosum* WASPS IN WIND TUNNEL WHEN EXPOSED TO VARIOUS STIMULI

Parameter	Stimulus				
	Clean air	Sex pheromone *Heliothis zea*	Sex pheromone *Spodoptera frugiperda*	Acetate blend	P^a
Number of observations	100	100	100	100	
Latency to takeoff (sec)	42.9 ± 5.8[b]	29.1 ± 4.1	35.3 ± 5.2	39.0 ± 5.4	n.s.
Individuals landing on platform (%)	17.0 ± 11.2[c]a	46.0 ± 18.5b	13.0 ± 5.1a	13.0 ± 5.1a	**
Individuals landing on upwind half of platform (%)	38.4 ± 7.4[b] (17)[d] [n.s.][e]	50.9 ± 5.8 (46) [n.s.]	48.3 ± 15.7 (13) [n.s.]	33.3 ± 16.3 (13) [n.s.]	n.s.

[a] n.s.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$.
[b] Mean ± SE. Differences tested with Kruskal-Wallis test (STSC, 1986).
[c] Mean ± SD of five blocks of 20 observations. Different letters indicate significant differences (95% LSD intervals) (STSC, 1986).
[d] In parentheses: number of landings of which position was recorded.
[e] In brackets: two-tailed significance level of difference from 50% (sign test).

of *T. pretiosum* when exposed to clean air vs. various concentrations of the pheromones of *H. zea* and *S. frugiperda*, respectively. With increasing concentrations of the sex pheromone of *H. zea* we observed a significant increase in total residence and walking times (Table 3). Other parameters (estimated path length, number of different sectors traversed) also showed an upward trend, but means were not significantly different. Furthermore, all parameters differed significantly from values obtained in clean air, as followed from paired tests with the lowest dosage. In contrast, dose–response relationships with *S. frugiperda* were insignificant for all parameters, except for walking velocity, which was higher with the highest dosage tested (Table 4). This value was also higher than the value obtained in clean air.

Similarly, in flight experiments we also found a significant dose response with the sex pheromone of *H. zea* (Table 5) and not with that of *S. frugiperda* (Table 6). With the pheromone of *H. zea*, increasing dosages yielded increasing percentages of wasps landing on the platform, and the value obtained with the lowest dosage (0.1 mg) was significantly different from that found in clean air. In contrast, the concentration of pheromone of *S. frugiperda* did not affect the percentage of wasps landing. With both odors, time until takeoff and the distribution of landings over upwind and downwind halves of the platform were

TABLE 3. BEHAVIOR OF INDIVIDUAL *Trichogramma pretiosum* WASPS ON PLATFORM IN WIND TUNNEL WHEN EXPOSED TO DIFFERENT DOSAGES OF SYNTHETIC SEX PHEROMONE OF *Heliothis zea*

Parameter	Clean air	Sex pheromone dosage (mg)			P^a
		0.1	1	10	
Number of observations	100	100	100	100	
Total time on platform (sec)	97.2 ± 13.9[b]	117.2 ± 13.4a (***)[c]	132.0 ± 16.6a	174.3 ± 17.5b	*
Time spent walking (%)	68.7 ± 2.3	87.4 ± 1.5	86.1 ± 1.8	85.8 ± 1.8	n.s.
Path length (number of sectors traversed)	15.0 ± 1.7	21.4 ± 2.0	22.9 ± 2.8	31.8 ± 3.3	n.s.
Walking velocity (number of sectors traversed/sec)	0.40 ± 0.03	0.26 ± 0.01	0.26 ± 0.01	0.25 ± 0.01	n.s.
Number of different sectors traversed	9.8 ± 0.6	12.6 ± 0.6	12.6 ± 0.6	14.2 ± 0.6	n.s.

[a] Kruskal-Wallis test for differences between dosages (i.e., clean air not included), followed by distribution-free multiple comparison. Different letters in a row indicate significant differences, n.s.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

[b] Mean ± SE.

[c] In parentheses: two-tailed significance level of difference with clean air (Mann-Whitney U test).

TABLE 4. BEHAVIOR OF INDIVIDUAL *Trichogramma pretiosum* WASPS ON PLATFORM IN WIND TUNNEL WHEN EXPOSED TO DIFFERENT DOSAGES OF SYNTHETIC SEX PHEROMONE OF *Spodoptera frugiperda*

Parameter	Clean air	Sex pheromone dosage (mg) 0.1	1	3	P^a
Number of observations	50	50	50	50	
Total time on platform (sec)	99.9 ± 15.6[b]	85.6 ± 14.1	101.0 ± 15.3	108.7 ± 17.9	n.s.
Time spent walking (%)	71.5 ± 3.1	61.6 ± 3.3	72.5 ± 2.9	64.4 ± 3.5	n.s.
Path length (number of sectors traversed)	17.9 ± 2.8	14.5 ± 2.3	15.6 ± 1.6	21.2 ± 3.1	n.s.
Walking velocity (number of sectors traversed/sec)	0.33 ± 0.02	0.40 ± 0.04 (n.s.)[c]ab	0.35 ± 0.02 (n.s.)a	0.40 ± 0.02 (***)b	*
Number of different sectors traversed	11.2 ± 0.9	9.4 ± 0.8	10.9 ± 0.8	12.1 ± 0.9	n.s.

[a] Kruskal-Wallis test for differences between dosages (i.e., clean air not included), followed by distribution-free multiple comparison. Different letters in a row indicate significant differences. n.s.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.
[b] Mean ± SE.
[c] In parentheses: two-tailed significance level of difference with clean air (Mann-Whitney U test).

TABLE 5. FLIGHT BEHAVIOR OF *Trichogramma pretiosum* WASPS IN WIND TUNNEL WHEN EXPOSED TO DIFFERENT DOSAGES OF SYNTHETIC SEX PHEROMONE OF *Heliothis zea*

Parameter	Clean air	Sex pheromone dosage (mg) 0.1	1	10	P^a
Number of observations	150	150	150	100	
Latency to takeoff (sec)	40.7 ± 5.2	35.2 ± 4.3	36.6 ± 2.6	36.5 ± 5.5	n.s.[b]
Individuals landing on platform (%)	16.3 ± 4.8	38.9 ± 3.9a (****)[d]	43.4 ± 6.8a	64.0 ± 9.7b	**[c]
Individuals landing on upwind half of platform (%)	37.5 ± 26.5 (6)[e] [n.s.][h]	56.3 ± 1.5 (18) [n.s.]	58.2 ± 3.1 (21) [n.s.]	—[f]	n.s.[g]

[a] n.s.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$.
[b] This row: mean ± SE. Kruskal-Wallis test of differences between pheromone dosages (i.e., clean air not included) (STSC, 1986).
[c] This row: mean ± SD of blocks of 20–25 observations. Different letters indicate significant differences between pheromone treatments (95% LSD intervals).
[d] In parentheses: two-tailed significance level of difference with clean air, t-test (STSC, 1986).
[e] In parentheses (this row): number of landings of which position was recorded.
[f] Not recorded.
[g] As 2, but Mann-Whitney U test.
[h] In brackets: two-tailed significance level of difference from 50% (sign test).

TABLE 6. FLIGHT BEHAVIOR OF *Trichogramma pretiosum* WASPS IN WIND TUNNEL WHEN EXPOSED TO DIFFERENT DOSAGES OF SYNTHETIC SEX PHEROMONE OF *Spodoptera frugiperda*

Parameter	Clean air	Sex pheromone dosage (mg) 0.1	1	3	P^a
Number of observations	200	100	100	100	
Latency to takeoff (sec)	39.3 ± 3.7	52.4 ± 7.6	35.3 ± 5.2	36.7 ± 4.8	n.s.[b]
Individuals landing on platform (%)	14.0 ± 8.6	14.0 ± 7.3a	13.0 ± 5.1a	13.0 ± 2.4a	n.s.[c]
Wasps landing on upwind half of platform (%)	48.5 ± 7.0 (28)[d] [n.s.][e]	44.0 ± 20.7 (14) [n.s.]	48.3 ± 15.7 (13) [n.s.]	20.0 ± 7.3 (13) [n.s.]	n.s.

[a] n.s.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$.
[b] This row: mean ± SE. Kruskal-Wallis test of differences between pheromone dosages (i.e., clean air not included) (STSC, 1986).
[c] This row: mean ± SD of blocks of 20–25 observations. Different letters indicate significant differences between pheromone treatments (95% LSD intervals).
[d] In parentheses (this row): number of landings of which position was recorded.
[e] In brackets: two-tailed significance level of difference from 50% (sign test).

not affected by odor concentration, and the percentage of wasps that landed on the upwind side did not differ significantly from 50%.

DISCUSSION

The two questions posed at the onset of this study were (1) does the response of *T. pretiosum* to *H. zea* sex pheromone represent a general response to any blend of aliphatic compounds, and (2) could it be a general response due to the artificial environment rather than a host-directed response. Both have been answered negatively. *Trichogramma pretiosum* apparently discriminated between different odors, since it did not respond to the sex pheromone of *S. frugiperda* or to a blend of saturated acetates. This differential response was probably not due to the dosage tested, as we found a clear dose effect with regard to total residence and walking times with *H. zea* and not with *S. frugiperda*. These results correspond with the fact that *H. zea* is a common field host of *T. pretiosum*, while eggs of *S. frugiperda* are very rarely parasitized by this species (Pinto et al., 1986). The fact that *T. pretiosum* does not use fall armyworm sex pheromone as a kairomone seems adaptive: eggs of this moth are difficult to parasitize because they are laid in batches covered by a thick layer of scales (Sparks, 1979) which seem to obstruct the parasitoid (Vickery,

1929; W.J. Lewis, personal observations). The scelionid *Telenomus remus* Nixon does attack *S. frugiperda* in the field (Wojcik et al., 1976). It is more robust than *T. pretiosum* and has been reported to dig successfully through the layer of scales of *Spodoptera litura* (F.) to reach the eggs (Braune, 1982). The same tactic may be used to attack *S. frugiperda*. Alternatively, *T. remus* may be aided by its distally positioned ovipositor (typical for scelionids), which facilitates probing through the layer of scales, while *T. pretiosum* cannot do so because (as in most chalcidoids) its ovipositor is ventrally located (M.R. Strand, personal communication). As in *T. pretiosum*, host acceptance in *T. remus* corresponds with responses to host sex pheromone: it was found to respond to an extract of abdominal tips of *S. frugiperda*, as well as to Z9–14:Ac and Z9–12:Ac, components of its sex pheromone (Nordlund et al., 1983).

Trichogramma spp. have been considered generalists in their choice of hosts (Hase, 1925; Salt, 1934; Sweetman, 1958; Thomson and Stinner, 1989). It has been suggested that plant-generalist natural enemies use more general chemical cues than plant specialists (Vinson, 1976; Sheehan, 1986). By this reasoning one might expect host-generalist parasitoids to use a wider range of chemical cues in their search for hosts than host specialists (Jones, 1986). Our study shows that *T. pretiosum* discriminated between blends of organic chemicals, which—although they differed in several aspects—were all straight-chain even-carbon-numbered aliphatic compounds. Similarly, *T. minutum* responded to a hexane extract of scales of *Choristoneura fumiferana* (Clemens) and not to an extract of *Sitotroga cerealella* (Oliver) scales (Zaborski et al., 1987). Although labeling organisms as generalist or chemical compounds as general seems rather arbitrary, these findings corroborate recent findings that species and/or strains of *Trichogramma* may be much more specific in their search for hosts and their use of semiochemicals than previously thought (van Dijken et al., 1986; Pak, 1988).

From the present results we cannot conclude what caused *T. pretiosum* to discriminate between the three odors. The blends differed in ratio of components, which in turn varied in chain length, presence of double bonds, and functional group. As far as the pheromonal function of these substances is concerned, each of these aspects can confer response specificity (Tumlinson and Teal, 1987). With regard to kairomonal effects, we can only speculate, for instance, that *T. pretiosum* might respond specifically to aldehydes and *T. remus* to acetates. The question of specificity also can be applied to parts of pheromone blends. Is the whole blend essential for a response, or is one component sufficient? In previous olfactometer experiments, *T. pretiosum* responded to the sex pheromone of *H. zea* released by calling moths (Noldus, 1988). However, we do not know which substance was responsible for that response. Noldus and van Lenteren (1985) found that *T. evanescens* did not respond to Z11–16:Ac, the major component of the sex pheromone of *M. brassicae*, in olfactometer

experiments with material tested at a single dosage. The concentration may have been incorrect, or a minor component (or combination of components) may be responsible for *Trichogramma*'s response to the odor of calling female moths. Obviously, additional studies are necessary to answer these questions.

Testing odors against clean air in a wind tunnel may not be the optimal assay for understanding how wasps use chemical cues during foraging in a chemically variable environment. Jones (1986) therefore urges experiments in more natural settings, where the significance of stimuli is studied by subtracting them from a "chemically complete" environment rather than adding them to a "chemically depleted" environment. However, the minute size of *Trichogramma* complicates direct observations of behavior under natural conditions. As a compromise, responses to kairomones can be studied in the laboratory in the presence of plants and other host-related cues. Results of recent experiments following this approach are reported elsewhere (Noldus et al., 1990b).

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